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\_\_\_\_\_ CTRS \_\_\_\_\_  
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\_\_\_\_\_ M903 \_\_\_\_\_  
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\_\_\_\_\_ NFDR \_\_\_\_\_  
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\_\_\_\_\_ C.AD \_\_\_\_\_  
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**Internal**

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Examiner Search Notes

\_\_\_\_\_ CLMPTO \_\_\_\_\_  
PTO Prepared Complete Claim Set

\_\_\_\_\_ ECBOX \_\_\_\_\_  
Evidence Copy Box Identification

\_\_\_\_\_ WCLM \_\_\_\_\_  
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\_\_\_\_\_ WFEE \_\_\_\_\_  
Fee Worksheet

**File Wrapper**

\_\_\_\_\_ FWCLM \_\_\_\_\_  
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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US93/00829 (22) International Filing Date: 5 February 1993 (05.02.93) (30) Priority data: 07/832,527        7 February 1992 (07.02.92)    US (71) Applicant: SYNTEX (USA) INC. [US/US]; 3401 Hillview Avenue, Palo Alto, CA 94304 (US). (72) Inventors: SUPERSAXO, Andreas, W. ; Gellert Str. 32, CH-4051 Basle (CH). KOU, Jim, H. ; 108 Mozart Street, Los Gatos, CA 95030 (US). (74) Agents: CHOW, Y., Ping et al.; Syntex (U.S.A.) Inc., 3401 Hillview Avenue, Palo Alto, CA 94304 (US).		(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  Published With international search report.																																									
(54) Title: CONTROLLED DELIVERY OF PHARMACEUTICALS FROM PREFORMED POROUS MICROPARTICLES <div style="float: right; text-align: right;">           ○ Experiment 1            ● Experiment 2            ▼ Experiment 7            ▼ Experiment 6         </div> <table border="1"> <caption>Estimated data from the graph</caption> <thead> <tr> <th>Days</th> <th>Experiment 1 (○)</th> <th>Experiment 2 (●)</th> <th>Experiment 7 (▼)</th> <th>Experiment 6 (▼)</th> </tr> </thead> <tbody> <tr><td>0</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td></tr> <tr><td>5</td><td>0.65</td><td>0.45</td><td>0.25</td><td>0.15</td></tr> <tr><td>10</td><td>0.75</td><td>0.55</td><td>0.28</td><td>0.20</td></tr> <tr><td>20</td><td>0.80</td><td>0.60</td><td>0.30</td><td>0.25</td></tr> <tr><td>30</td><td>0.82</td><td>0.62</td><td>0.32</td><td>0.28</td></tr> <tr><td>40</td><td>0.83</td><td>0.63</td><td>0.33</td><td>0.30</td></tr> <tr><td>50</td><td>0.84</td><td>0.64</td><td>0.34</td><td>0.32</td></tr> </tbody> </table>				Days	Experiment 1 (○)	Experiment 2 (●)	Experiment 7 (▼)	Experiment 6 (▼)	0	0.0	0.0	0.0	0.0	5	0.65	0.45	0.25	0.15	10	0.75	0.55	0.28	0.20	20	0.80	0.60	0.30	0.25	30	0.82	0.62	0.32	0.28	40	0.83	0.63	0.33	0.30	50	0.84	0.64	0.34	0.32
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**CONTROLLED DELIVERY OF PHARMACEUTICALS FROM PREFORMED  
POROUS MICROPARTICLES**

**BACKGROUND OF THE INVENTION**

5     **a) Field of the Invention**

          This invention pertains to controlled release  
pharmaceutical compositions, processes for preparing the  
compositions, and uses thereof.

10

**b) Description of Related Art**

          The advent of genetic engineering has resulted in a  
proliferation of new biopharmaceuticals that are orally  
15     inactive and must be administered by subcutaneous injection  
or intravenous infusion. Considerable effort has been  
invested in techniques, e.g. passive transdermal delivery,  
iontophoresis, and permeability enhancement, that have the  
potential to overcome the barriers presented by natural  
20     biological membranes and avoid the trauma of injections.  
Some successes with nasal, rectal, transdermal and  
gastrointestinal administration have been achieved with lower  
molecular weight polypeptides. However, parenteral  
administration remains the only viable route by which  
25     sustained, controlled delivery can be achieved.

          The susceptibility of peptides and proteins to  
proteolysis and rapid clearance from the bloodstream makes  
them ideal candidates for controlled delivery systems.  
In addition, due to the high molecular weight and  
30     hydrophilicity of proteins, transdermal diffusion is  
impractically slow. Furthermore, the zero-order delivery  
kinetics associated with most transdermal systems may not

be the optimum kinetic profile because of the potential down-regulation of biological receptors; in many cases pulsed or self-regulated delivery systems may be more efficient and economical.

5        One class of polymer systems for controlled release of polypeptides is based on polyethylene-co-vinyl acetate (EVA). Langer et al. in *Nature* 263, pp. 797-800 (1976) have shown that a wide variety of water-soluble macromolecules can be released for weeks and months from thin EVA matrices, formed  
10 by suspending macromolecular drug powder in an organic polymer solution and evaporating the solvent. Microscopy of these polymer matrices revealed a two-phase dispersion, with domains of solid drug and polymer matrix. Mechanistic studies have suggested that the macromolecules are released from  
15 these devices by a self-diffusion process, i.e. release does not involve dissolution of the drug in EVA or swelling of the polymer bulk, but rather diffusion through aqueous channels and pores created by the dissolution of dispersed macromolecules.

20        The pioneering work of Kent et al., as disclosed in U.S. Patent No. 4,675,189, provided for microencapsulation of biologically active substances in polymeric matrices. Since that invention, however, little progress has been made in the development of second generation systems designed to  
25 provide controlled release of a variety of active agents.

#### SUMMARY OF THE INVENTION

30        A controlled release pharmaceutical composition comprising a physiologically active agent dispersed in preformed porous polymeric microparticles is provided. The active agent concentration may be up to about 10% by weight to achieve controlled release. In some instances the  
35 concentration of active agent must be no greater than about 1% by weight for obtaining the desired controlled release.

Each of the porous microparticles has a plurality of

preformed pores into which the active agent is loaded and from which the active agent is subsequently released to the environment of use. The compositions are capable of delivering physiologically effective amounts of active agent for at least about thirty days.

The active agent is preferably one which is effective at the microgram, nanogram, or picogram daily level. Optionally, the compositions are implanted at the situs of use.

In one embodiment the microparticles are polymers of polyanhydride, polylactic, polyglycolic, or copoly(lactic/glycolic) acid and the active agent is a polypeptide, preferably a highly potent polypeptide, most preferably a LHRH analog, neurotrophic factor or an interleukin.

In a process for preparing the pharmaceutical compositions, the preformed porous microparticles are suspended in a solution of the active agent. Optionally, vacuum or pressure may be applied to facilitate migration of the active agent into the microparticles. After the active agent has deposited in the microparticles, they are dried, and further processed as required to obtain a stable, biologically active pharmaceutical composition.

Also provided is a process for the controlled delivery of a physiologically effective amount of an active agent to an animal or human subject by introducing preformed porous microparticles containing from about 0.0001% to about 10% by weight of active agent into the subject. In some instances the amount of active agent is from about 0.0001 to about 1.0% by weight.

The release of active agent from the microparticles may be controlled by exposure to ultrasonic radiation. This ultrasonic stimulation is reversible and may be used to provide pulsatile delivery of the active agent in situ.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1a and 1b show the cumulative percent release and mass release rate [ng/mg microspheres/day] profiles, respectively, for dextran-containing microparticles at various loading levels.

Figure 2 compares the cumulative percent release from microparticles containing dextran plus an excipient (sucrose) to that of microparticles containing dextran only.

Figure 3 shows the effect of ultrasound on the mass release rate [ng/mg microspheres/day] of dextran from the microparticles.

Figure 4 compares the daily release rate [% of total/day] of mannitol from the microparticles as determined *in vivo* to that determined *in vitro*.

Figures 5 and 6 show the cumulative fractional release and mass release [ $\mu$ g/100mg microspheres/day] profiles, respectively, for nafarelin-containing microspheres at various loading levels.

Figure 7 shows the effect of release-modifying additives on the cumulative fractional release profiles of nafarelin-containing microparticles.

### DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "physiologically active agent" refers to a substance useful for the diagnosis, treatment or prevention of a human or animal disorder, or in the regulation of a human or animal physiological condition or metabolic state.

As used herein, the term "polypeptide" refers to a physiologically active agent whose active portion constitutes an amino acid sequence of varying length from about two amino acids to hundreds of amino acids, which are often referred to as peptides and proteins. Generally, the molecular weight of the polypeptides ranges between 200 and 100,000. The active portion may also contain additional derivatizing groups such as sugars or lipids.

Suitable polypeptides are exemplified by, for example,

insulin, proinsulin, glucagon, parathyroid hormone, calcitonin, vasopressin, erythropoietin (EPO), renin, prolactin, human growth hormone (hGH), thyroid-stimulating hormone (TSH), corticotropin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), chorionic gonadotropin, atrial peptides, interferon, tissue plasminogen activator (TPA), gamma-globulin, factor VIII, urokinase, streptokinase, the various lymphokines such as the interleukins (I, II, III, IV, V, VI, and VII), colony stimulating factors, and so forth. As is well known, modifications of the native amino acid sequences of the foregoing polypeptides and fragments thereof may also be used as agonists or antagonists for the native polypeptides.

Examples of native polypeptides for which altered or fragmented agonists or antagonists are available include growth hormone releasing factor, corticotropin releasing factor, luteinizing hormone releasing hormone (LHRH), somatostatin, calcitonin, thyrotropin releasing hormone, calcitonin gene related peptide (CGRP), and proteins such as enzymes, including transferases, hydrolases, isomerases, proteases, ligases, oxidoreductases, esterases, phosphatases, and various growth and neurotrophic factors, such as nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor, tumor necrosis factor (TNF) and transforming growth factor (TGF). Other proteins include vaccines derivable from the proteins of viral and bacterial and parasitic infective agents.

By utilizing the method of the invention, it is possible to prepare pharmaceutical compositions suitable for parenteral administration which contain endogenous opioid agonists, such as enkephalins and endorphins; hypothalamic hormones, such as gonadoliberin, melanostatin, melanoliberin, somatostatin, thyroliberin, substance P, and neurotensin; adenohipophyseal hormones, such as corticotropin, lipotropin,



melanotropin, lutropin, thyrotropin, prolactin, and somatotropin; neurohypophyseal hormones; calcitropic (thyroid) hormones, such as parathyrin and calcitonin; thymic factors, such as thymosin, thymopoietin, circulating thymic factor, and thymic humoral factor; pancreatic hormones, such as insulin, glucagon and somatostatin; gastrointestinal hormones, such as gastrin, cholecystokinin, secretin, gastric inhibitory polypeptide, vasointestinal peptide, and motillin; chorionic (placental) hormones, such as choriogonadotropin and choriomammotropin; ovarian hormones, such as relaxin; vasoactive tissue hormones, such as angiotensin and bradykinin; growth factors, such as somatomedins, epidermal growth factors, urogastrone, and nerve growth factor; hemophilia factors, such as blood clotting factors VIII and IX; enzymes, such as streptokinase, fibrinolysin, deoxyribonuclease, and asparaginase; and artificial or pseudo peptides, such as deferoxamine. Many other classes and specific types of peptide and protein hormones and other biologically active molecules are known, such as the LHRH analogs buserelin, deslorelin, gonadorelin, goserelin, histrelin, leuporelin, nafarelin, or triptorelin. Peptide and protein hormones suitable for use in the present invention are disclosed in Johannes Meienhofer, "Peptide and Protein Hormones", in *Burger's Medicinal Chemistry*, 4th ed., (part II), Wolff, Ed., John Wiley and Sons (1979).

The listing of certain polypeptides in this application is not intended to be exclusive, and it may easily be determined by conventional release studies if a particular polypeptide has sufficient biological activity to be used. A physiologically effective amount of the polypeptide is the amount of polypeptide required to produce the effect ordinarily associated with administration of that polypeptide. Typically, the polypeptide should be active at the microgram to picogram daily level. That is, the polypeptide is effective for its intended purpose when microgram to picogram amounts are delivered per day. Such polypeptides are characterized as "highly potent".

For example, the highly potent LHRH agonist, nafarelin, is active at about 24 micrograms/day dosing. A 100 mg bolus (i.e., given rapidly at one time for immediate response) of microparticles containing about 1.0 mg of nafarelin (1% loading) could deliver 24 micrograms/day of nafarelin for about 40 days. A 40 mg bolus of microparticles containing about 1.0 mg of nafarelin (2.5% loading) could deliver greater than 10 micrograms/day of nafarelin for about 50 days.

The term "controlled release" as used herein is meant to describe the phenomenon whereby the active agent is released from the microparticles to the environment of use over an extended period of time. In many instances the release profile is characterized by an initial burst, in which as much as 50% of the active agent is released in a matter of hours, followed by an essentially constant, slow release of active agent from the microparticles.

For some drugs, high initial burst may be desirable in order to induce a rapid, intense physiological response, followed by a period of maintenance therapy; for other drugs, low (<10%) initial bursts are desirable to minimize side effects and avoid initial hyperactivity, while still providing a physiologically effective dose over time. The latter objective may be achieved by use of low loading level microparticles, having in the order of 1 to 2 weight percent active agent.

The release rate may also be controlled to some extent by the co-incorporation of release rate modifying excipients and additives, generally in an amount less than the active agent. Additionally, in the event the active agent is one which is deactivated by freeze drying, a cryoprotectant may be added. Generally, the amount of cryoprotectant greatly exceeds the amount of active agent.

Suitable excipients, additives and cryoprotectants include proteins, such as serum albumin; carbohydrates, including simple sugars such as mannitol and sucrose; or polysaccharides such as dextran; lipids such as 1,2-di-

palmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-sn-glycero-3-[phospho-rac-(1-glycerol)]sodium salt (DPPG), and mixtures thereof; or surfactants such as Polysorbate 80 (Tween 80).

5           The cryoprotectant and the release rate controlling excipient or additive need not be the same, but may be independently selected. Selection of an appropriate excipient, additive or cryoprotectant is within the purview of the skilled artisan; representative compounds may be found  
10   in *Remington's Pharmaceutical Sciences*, 18th Ed., (Mack Publishing Company, Easton, PA, 1990). An important feature of this invention is the use of preformed porous microparticles for delivery of the active agent. The microparticles, which may assume a variety of shapes, generally  
15   have diameters of from about 50 to about 400 microns and are extensively permeated with a network of pores into which the active agent is introduced. In a preferred embodiment, the microparticles are microspheres. Pore sizes range from about 0.01 to about 1.0 micron in diameter with no particular  
20   distribution of shape or size required. The void volume of the microparticles may range from 20 to 90%, preferably from 30 to 80%, most preferably from 50 to 80%.

          The microparticles are prepared from any suitable polymeric material, such as polyesters, polyamides, poly-  
25   anhydrides, and polyacrylates. Preferably, the polymer is one which will degrade over time in the body, such as poly-(lactic) acid, poly(glycolic) acid, copoly(lactic/glycolic) acid, and poly[1,3-bis(p-carboxyphenoxy)propane-co-sebacic acid]. Processes for forming such particles will be apparent  
30   to the skilled artisan and include, but are not limited to, spray drying of the polymeric material to generate substantially spherical particles or freeze drying followed by ball milling to produce randomly shaped particles. Unlike the microencapsulation processes of the art which rely upon  
35   simultaneous formation of the microparticle and incorporation of the active agent, the current invention starts with preformed porous microparticles and adds the active agent in

a separate step. This approach has the advantage of avoiding exposure of the active agent to potentially denaturing solvents, temperatures, or other reaction conditions.

5 The active agent may be induced to migrate into the microparticles by simply equilibrating the microparticles in a solution or suspension having the desired concentration of the active agent. Suitably, the active agent may migrate into the microparticles over a period of about 15 min to about 30 hours. Either aqueous or non-aqueous solvents such as  
10 methanol, ethanol or iso-propanol may be advantageously used depending upon the choice of active agent and microparticle type. Alternatively, more aggressive techniques, such as the application of pressure or vacuum, may be employed to accelerate the migration of the active agent into the micro-  
15 particles. Optionally, the microparticles may be pretreated to increase their compatibility with the active agent; for example in the case of poly(lactic) acid microparticles, it is advantageous to pre-wet them with ethanol.

After adding the active agent, the microparticles may be  
20 dried in air, under vacuum, by controlled evaporative drying, by a flowing inert gas, by freeze drying, or by such other techniques as will be apparent to the skilled artisan. Preferably, the active agent concentration in the microparticles is from about 0.0001 to about 10 weight  
25 percent, more preferably from about 0.001 to about 5 weight percent. Another range of active agent concentrations is from about 0.0001% to about 1.0% weight percent, more preferably from about 0.001 to about 0.5 weight percent.

It is particularly surprising that in some instances  
30 controlled release for long time periods may be achieved by the incorporation of lesser, rather than greater, amounts of active agent.

One advantage of the invention is that the dried, active agent-containing microparticles may be stored indefinitely  
35 without adverse effects on the potency of the active agent. When it is desired to administer the microparticles, they may be suspended in a suitable pharmaceutical medium and

administered to the subject. In one embodiment, the microparticles are implanted at the situs where the physiological effect is desired, for example, in a neoplasm.

5 Porous microparticles which are suitable for use in the invention include, but are not limited to, Accurel® microporous polymer products (Akzo Fibers and Polymers Division, Accurel Systems, Box D-8753, Obernburg, Germany). These microparticles are microspheres having diameters of about 100 to 300 microns, void volumes of 50 to 80 %, pore  
10 sizes of 0.1 to 1 micron, and a loading capacity of up to 70 % by weight. The preparation of these materials is described in U. S. Patent No. 4,645,664, issued February 24, 1987.

Typically, it is stated there that the microspheres are  
15 prepared by dissolving finely divided polylactide (homopolymer of L-lactic acid) in phthalic acid diethyl ester. The components are mixed with stirring and heating, preferably at a temperature of about 150° to 170° for 1 to 2 hours. In case insoluble impurities are present, the hot  
20 solution is filtered. The clear solution is then slowly cooled, e.g., by allowing it to stand at room temperature. The resulting suspension consists of a crumbly mass which is collected by filtration and still contains phthalic acid diethylester depending on the concentration and quality of  
25 the polylactide used. The crumbly mass is extracted with a solvent suitable to dissolve the phthalic acid diethylester but not the polylactide. Examples of such solvents are methanol, ethanol, and isopropanol; ethanol being the preferred solvent. It is advantageous to carry out the  
30 extraction in an extraction apparatus such as a Soxhlet apparatus. The solid is collected and dried at 40 to 60° and then converted into powdery form, e.g., by passing it through a screen or mesh.

### 35 Administration

The active agent-containing microparticles of this invention can be easily administered in various dosage forms

as injections and implants intramuscularly, subcutaneously, intravenously, or at an organ, joint cavity, or at a lesion.

For example, an injectable formulation of the micro-  
particles of this invention may be dispersed in a suitable  
5 aqueous medium, optionally containing preservatives (e.g. methylparaben) and/or isotonicizing agents (e.g. sodium chloride, sorbitol) or they may be suspended in an aqueous medium together with a vegetable oil (e.g. sesame oil). The  
10 dose of the controlled release composition of this invention and the selection of suitable adjuvants, carriers, and solvents will be affected by the contemplated end use and will vary depending upon the nature and amount of physiologically active agent in the microparticles, the dosage form, the desired duration of release, the recipient  
15 animal, and the purpose of the administration. A single dose of microparticles may be in the range of from about 0.01 mg to about 100 mg/kg of body weight.

This invention also provides a process for modulating the release of active agent from the microparticles by  
20 exposing them to bursts of ultrasonic radiation. The release rate of active agent is increased by as much as three orders of magnitude by exposure to the radiation. When the irradiation is terminated, the release rate returns to pre-irradiation levels. This process may be applied in a cyclical  
25 fashion to provide a high dose of active agent at a particular time followed by constant low dosing until it is deemed desirable to again provide the higher dose. The method may be practiced by exposure of the situs where the microparticles are located to the ultrasonic radiation.

30

#### EXAMPLES

The following Examples are presented solely to  
35 illustrate representative embodiments of the invention and should not be construed as limiting the scope of the claims in any way.

**EXAMPLE 1. Dextran-Containing Microparticles**

1 g of Accurel® poly(L-lactic)acid (inherent viscosity 1.2, MP = 184°C) microspheres were wetted with 15 mL of 50% ethanol and washed three times with 15 mL of water. After  
5 decanting the water, the microspheres were equilibrated for 1 hour in 1 mL of aqueous solutions of [<sup>14</sup>C]-labelled dextran (MW = 10,200, 2mCi/g, Sigma) to obtain microspheres having 2.54, 1.06, and 0.42 wt% loading. The microspheres were then filtered and freeze-dried.

10 The amount of active agent incorporated per unit weight of microspheres was determined by scintillation counting of the labelled active agent-containing microspheres. 20 mg of labelled active agent-containing microspheres were dissolved in 2 mL of methylene chloride and 20 mL of scintillation  
15 cocktail (Ready-Safe, Beckmann Instruments, Inc.) and the active agent concentration determined by liquid scintillation spectrometry (Liquid Scintillation Counter LS 8100, Beckmann Instruments, Inc.).

In vitro release studies were performed by rotating a 50  
20 mg sample of the microspheres in 10 mL of phosphate buffered saline (PBS, pH 7.4) (Vanderkamp® Sustained Release Apparatus, VanKel Industries, Inc.). The receiving fluid was periodically withdrawn and the study continued with fresh buffer. The amount of active agent released was quantified by  
25 measuring the radioactivity.

For all three preparations an initial fast release (burst) was observed. At the highest loading, approximately 80% of the active agent was released during the burst phase. However, at lower loading levels, the burst was reduced and  
30 the remaining portion was released at essentially a constant rate over several months (Figs. 1a, 1b).

**EXAMPLE 2. Coincorporation of Rate-Modifying Excipients**

35 Following Example 1, microspheres containing 0.12 wt % dextran plus 0.28 wt % sucrose (0.40 wt % total) were prepared. Figure 2 compares the release of the dextran from

the dextran/sucrose microspheres to that from microspheres containing 0.42 wt % dextran only.

5 **EXAMPLE 3. Effect of Ultrasound**

50mg of Example 1 microspheres (0.42% dextran),  
preincubated in the rotating release apparatus described  
above, were periodically exposed for 5 min. to ultrasound.  
Vials containing the spheres were transferred into a glass  
10 beaker containing 250 mL water. The ultrasound device used  
was a Branson 2200 Ultrasonic Cleaner (Branson®, Branson  
Ultrasonics Corporation, Danbury, CT) generating a ultrasonic  
energy of  $\sim 0.3 \text{ W/cm}^2$  (personal communication, Branson  
Ultrasonics Corporation). After irradiation the microspheres  
15 were returned to the release apparatus. Figure 3 demonstrates  
that the release of active agent from the porous microspheres  
can be reversibly increased by nearly three orders of  
magnitude by exposing them to low energy ultrasound ( $\sim 0.3$   
 $\text{W/cm}^2$ ). The release rate (ng/mg spheres/day) at day eight  
20 increased from 31 before irradiation to 18500 during the  
exposure to ultrasound and decreased thereafter within 3 hr  
to 511. On day eleven, before the 2nd exposure to ultrasound  
the release rate was 22. The release rates during the 2nd and  
3rd (day thirteen) triggering periods were 17,300 and 11,000,  
25 respectively.

**EXAMPLE 4. In Vivo Release**

Following Example 1, microspheres containing 0.21 wt%  
30 D-[1- $^{14}\text{C}$ ] labelled mannitol (MW 182, 60 mCi/mmol, Amersham)  
were prepared. Three male rats (strain CD BR Vaf+) (180-300  
g) were anesthetized and 100 mg microparticles inserted with  
a trocar s.c. through a small surgical incision on the back  
of the neck. After implantation the rats were transferred to  
35 individual metabolic cages. Since mannitol is not metabolized  
and is excreted solely in the urine, release of the mannitol  
was monitored by measuring the amount excreted in the urine.



The amount of radioactive mannitol excreted in the urine was quantified by counting the radioactivity present in 0.5 mL of aqueous sample emulsified in 20 mL of liquid scintillation cocktail.

5       The in vivo release profile of mannitol from the microspheres is illustrated in Figure 4. After an initial burst, the remaining portion was released in a controlled manner. The release rates (% of total/day) gradually decreased from 27.0% +/- 12.5% at day one (burst) to 1.7% +/- 0.12% at day ten, 0.32% +/- 0.06% at day twenty, and 0.28% +/- 0.07% at day thirty. The implanted spheres were also tested for their in vitro release behavior and, as shown in Figure 4, a good correlation between in vitro and in vivo release profiles was found.

15

#### **EXAMPLE 5. Polypeptide-Containing Microparticles**

Following the procedure of Example 1, preformed porous microparticles containing up to 1.0% human growth hormone (hGH, MW  $\approx$  22,000) may be prepared. A 10 mg sample of such particles may be introduced into a subject and is expected to release about 500 nanograms of hGH per day for as long as 100 days.

25       Similarly, microparticles containing up to 1% of the LH-RH agonist nafarelin (MW = 1400) may be prepared and are expected to release physiologically effective amounts of active agent for several months.

#### **Example 6. Nafarelin-Containing Microparticles**

30       1 g of Accurel® poly(L-lactic)acid (inherent viscosity 1.2, MP = 184°C) microspheres were wetted with 15 mL of methanol and washed three times with 15 mL of water. The microspheres were equilibrated for 18 hours at 37° in 2 mL of a 20% methanol/ 80% water solution containing various concentrations of nafarelin acetate (Syntex Corp., Palo Alto, California). The loaded microspheres were

35

collected by filtration and dried in a vacuum oven at 40°.

#### Loading Determination

50 mg of loaded microspheres were sonicated and dissolved in  
 5 2 mL of 1:1 N-methylpyrrolidone/dimethylacetamide solution. A  
 100 µL portion of this solution was added to 4 mL of  
 acetonitrile and the mixture was made up to 10 mL with 0.1M  
 pH 7 sodium phosphate. The polymer was precipitated by the  
 addition of phosphate buffer. The solution was filtered  
 10 through a disposable 0.45 µm nylon 66 membrane filter. The  
 filtrate, after discarding the first 2 mL, was assayed for  
 nafarelin content by an isocratic reversed phase HPLC method  
 using a C<sub>8</sub> column. The mobile phase was 30% acetonitrile in  
 50mM pH 3 sodium phosphate. The flow rate was set at 1mL/min  
 15 and the temperature was set at ambient.

The compositions of various loading solutions are summarized  
 in Table I below. In experiments 1-3, different  
 concentrations of nafarelin acetate were used, whereas in  
 experiments 4-7 various excipients or additives such as  
 20 dextran (mw 9300), Tween 80, and lipids were added.

TABLE I. Compositions of Loading Solutions and Resulting  
 Loading Levels of Nafarelin Acetate in Porous Preformed  
 Polylactic Acid Microspheres

Exp.	Nafarelin (mg/mL)	Excipient/Additive (mg/mL)	Methanol (%)	Loading <sup>1</sup> (%) (s.d.)
1	100	---	20	7.10 (0.16)
30 2	10	---	20	2.43 (0.05)
3	2	---	20	1.41 (0.05)
4	10	10 (dextran)	20	2.27 (0.13)
5	10	100 (dextran)	20	2.70 (0.09)
6	10	10 (Tween 80)	20	4.10 (0.11)
35 7	50	58:2 (DPPC:DPPG)	0	8.04 (0.05)

<sup>1</sup>Numbers reported are means of three experiments with  
 s.d.'s (standard deviations) in brackets.

#### Drug Loading of Nafarelin

The results from experiments 1-3 show a correlation between the loading level and the nafarelin concentration in the loading range between 2 and 100 mg/mL. The loading does not increase proportionally with the concentration of the loading solution. Experiments 2, 4 and 5 show that dextran does not have a significant effect on the nafarelin loading. Comparisons of experiments 2 and 6 and 1 and 7 indicate that Tween 80 and lipids (DPPC/DPPG) increase the loading.

#### Reproducibility of Drug Loading

Three separate batches of microspheres were loaded individually using the loading solution of experiment 5 (Table I). The loading levels of these batches were determined to be 2.7%, 2.48% and 2.62%.

#### Example 7. In Vitro Release from Nafarelin-Containing Microparticles

100 mg of dry loaded microspheres were dispensed into a scintillation vial. 20 mL of fresh receiver fluid, 0.1M pH 7 sodium phosphate buffer containing 0.1% sodium azide, were added. The vial was closed with a Teflon cap equipped with a sampling port. The vial was then placed into a rack that was set into a tumbling motion. The apparatus was immersed in a constant temperature water bath at 37°C. The entire volume in the vial was sampled at fixed intervals and replaced with fresh 20 mL portions of receiver fluid. The samples were assayed by an isocratic reversed phase HPLC method using a C<sub>8</sub> column. The mobile phase was 30% acetonitrile in 50mM pH 3 sodium phosphate. The flow rate was set at 1mL/min and the temperature was set at ambient.

**Drug Release Profiles of Nafarelin from Microspheres**  
Figures 5-7 (experiments 1-7)

Experiments 1-3 (Figure 5) demonstrate that the drug  
5 release profiles of nafarelin are more or less biphasic, with  
a burst phase followed by an extended slow release phase. The  
one-day burst is less than 5%, 25% and 55% at the loading of  
1.4%, 2.4% and 7.1%, respectively.

Experiments 1-3 (Figure 6) further show that there is a  
10 correlation between the overall release profile of nafarelin  
and the loading level. The release rate of nafarelin was  
faster at higher loading levels. After 5 days the release  
rates fall into the range of 1 to 100  $\mu\text{g/day}/100\text{ mg}$  loaded  
microspheres; after about 20 days the release rate is in the  
15 range of 2 to 15  $\mu\text{g/day}$  from 100 mg loaded microspheres up to  
at least day 50.

Experiments 1, 2, 6 and 7 (Figure 7) show the effect  
additives such as lipids (DPPC/DPPG) or Tween 80, a non-ionic  
surfactant, have on the release profile of nafarelin from  
20 microspheres. The data suggest that both the lipids  
(DPPC/DPPG) and Tween 80 slow the release of nafarelin.

**Example 8. Neurotrophic Factor Containing Microparticles**  
25

Following the procedures of Example 1 and 6, preformed  
microparticles containing up to 5% nerve growth factor (NGF,  
MW  $\approx$  26,000) or ciliary neurotrophic factor (CNTF, MW  $\approx$   
22,000) may be prepared. A 10 microgram sample of such  
30 particles may be introduced into a subject and is expected to  
release physiologically effective amounts of the active agent  
for several months.

## WHAT IS CLAIMED IS:

1. A controlled release pharmaceutical composition comprising a physiologically active agent dispersed in preformed porous polymeric microparticles, wherein said  
5 active agent is present in an amount from about 0.0001% to about 10% by weight, each of said porous microparticles having a plurality of preformed pores into which active agent is loaded and from which active agent is released to the environment of use, said composition being capable of  
10 delivering physiologically effective amounts of active agent for at least about thirty days.
2. A composition of claim 1 wherein said active agent is present in an amount from about 0.001% to about 5% by  
15 weight.
3. A composition of claim 1 wherein said active agent is present in an amount from about 0.0001% to about 1.0% by weight.  
20
4. A composition of claim 1 wherein said microparticles are formed from polylactic acid, polyglycolic acid, copoly(lactic/glycolic)acid, or poly[1,3-bis(p-carboxy-  
25 phenoxy)propane-co-sebacic acid].
5. A composition of claim 1 wherein said active agent is an LHRH analog, or a neurotrophic factor.
6. A composition of claim 5 wherein said LHRH analog  
30 is buserelin, deslorelin, gonadorelin, goserelin, histrelin, leuprorelin, nafarelin, or triptorelin.
7. A composition of claim 6 wherein said LHRH analog is nafarelin.  
35
8. A composition of claim 5 wherein said neurotrophic factor is selected from brain derived neurotrophic factor,

ciliary neurotrophic factor, fibroblast growth factor, glial derived neurotrophic factor, and nerve growth factor.

9. A composition of claim 8 wherein said neurotrophic  
5 factor is ciliary neurotrophic factor or nerve growth factor.

10. A composition of claim 1 further comprising a  
release rate modifying additive, excipient or a  
cryoprotectant.

10

11. A composition of claim 1 wherein the microparticles  
are in the form of microspheres having diameters from about  
50 to about 400 microns.

15

12. A composition of claim 11 wherein the micro-  
particles comprise microspheres of poly(L-lactic) acid.

13. A controlled release pharmaceutical composition  
comprising from about 0.0001% to about 10% by weight of a  
20 physiologically active agent in preformed porous micro-  
particles of polylactic acid, polyglycolic acid, copoly-  
(lactic/glycolic) acid or poly[1,3-bis(p-carboxy-  
phenoxy)propane-co-sebacic acid], said composition being  
capable of delivering a physiologically effective amount of  
25 active agent over a period of at least about thirty days.

14. A controlled release composition of claim 13  
wherein said active agent is a polypeptide present in an  
amount from about 0.001% to about 5% by weight.

30

15. A controlled release composition of claim 13  
wherein said active agent is a polypeptide present in an  
amount from about 0.0001% to about 1.0% by weight.

16. A process for the controlled delivery of a  
35 physiologically effective amount of an active agent to an  
animal or human subject which process comprises introducing

preformed porous microparticles containing from about 0.0001% to about 10% by weight active agent into the subject.

17. A process of claim 16 wherein said preformed porous microparticles contain from about 0.001% to about 5% by weight active agent.

18. A process of claim 16 wherein said preformed porous microparticles contain from about 0.0001% to about 1.0% by weight active agent.

19. A process of claim 16 wherein the microparticles are implanted at a specific locus where the physiological effect is desired.

20. A process of claim 19 further comprising intermittent exposure of the microparticles to ultrasonic radiation thereby reversibly controlling the release of active agent from the microparticles.

21. A process for preparing a composition of claim 1 which process comprises:

a) mixing the porous microparticles with a solution or suspension containing an active agent for a period of time sufficient for the microparticles to absorb from about 0.0001% to about 10% by weight of the active agent from the solution or suspension; and

b) removing the solvent.

22. A process of claim 21 wherein in step (a) the amount of active agent is from about 0.001% to about 5% by weight.

23. A process of claim 21 wherein step (a) comprises suspending the porous microparticles in a solution containing a polypeptide for a period of time sufficient to absorb from about 0.0001% to about 1.0% by weight of the polypeptide.

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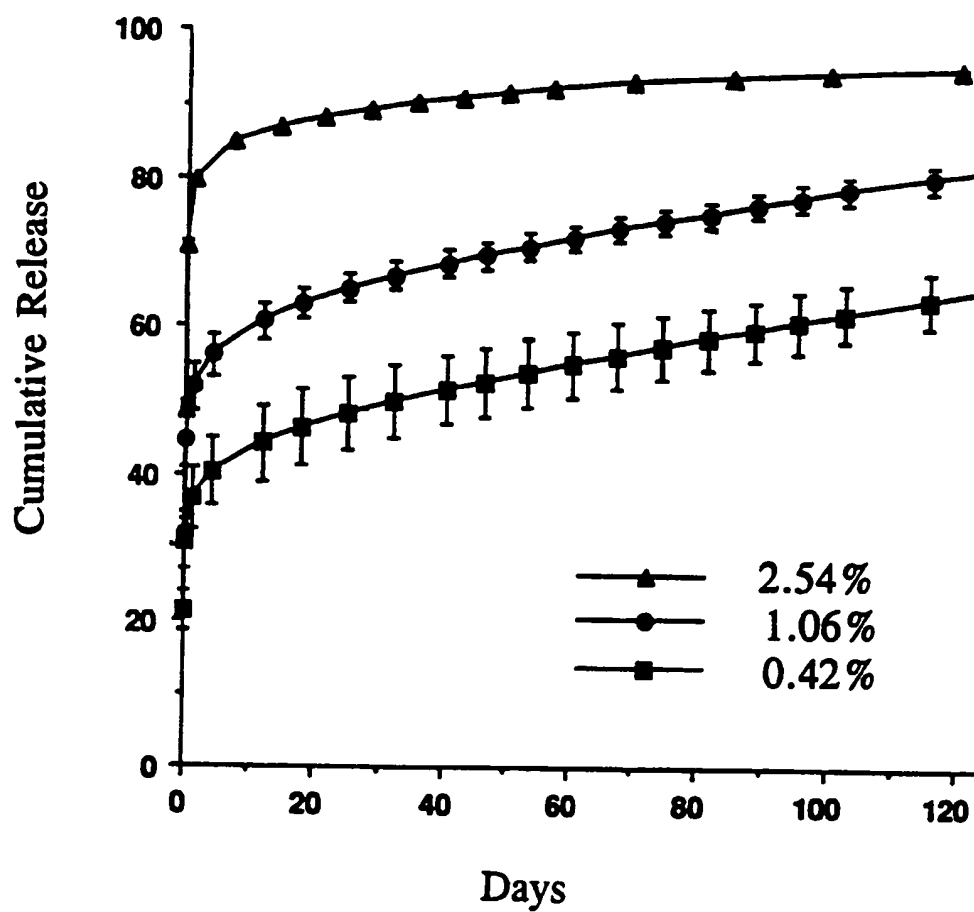


Figure 1a

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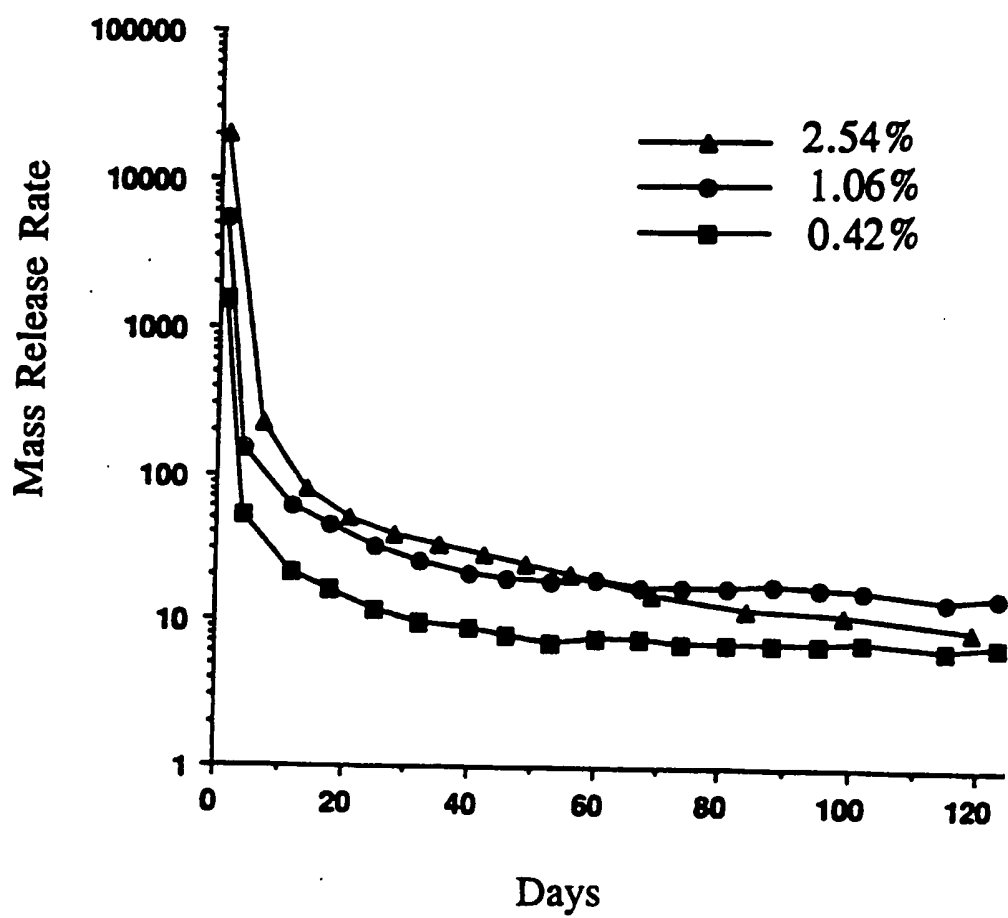


Figure 1b

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ISA/EP

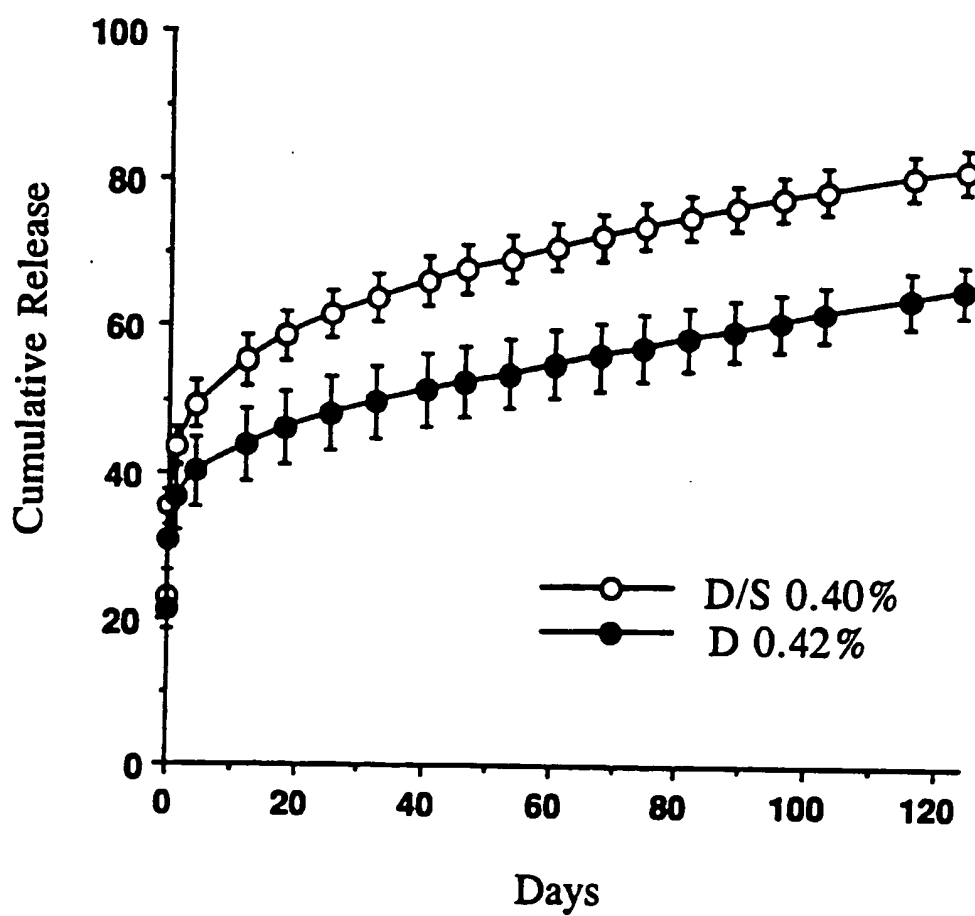


Figure 2

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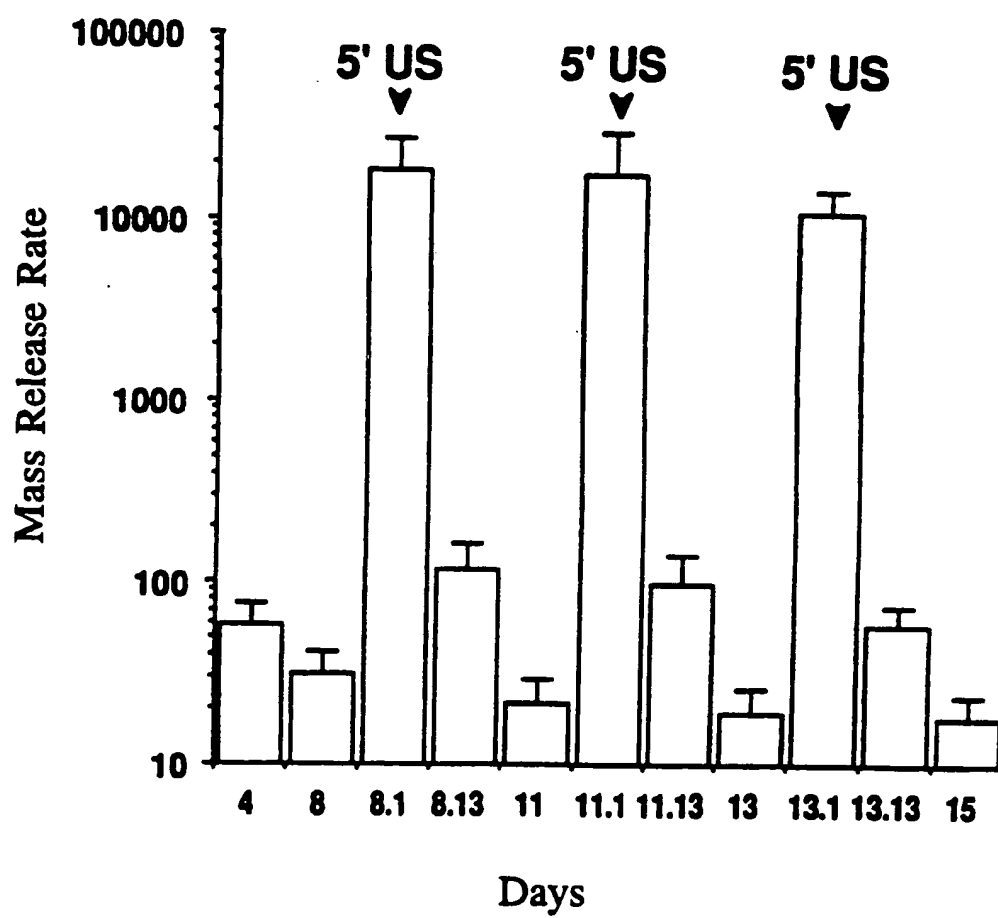


Figure 3

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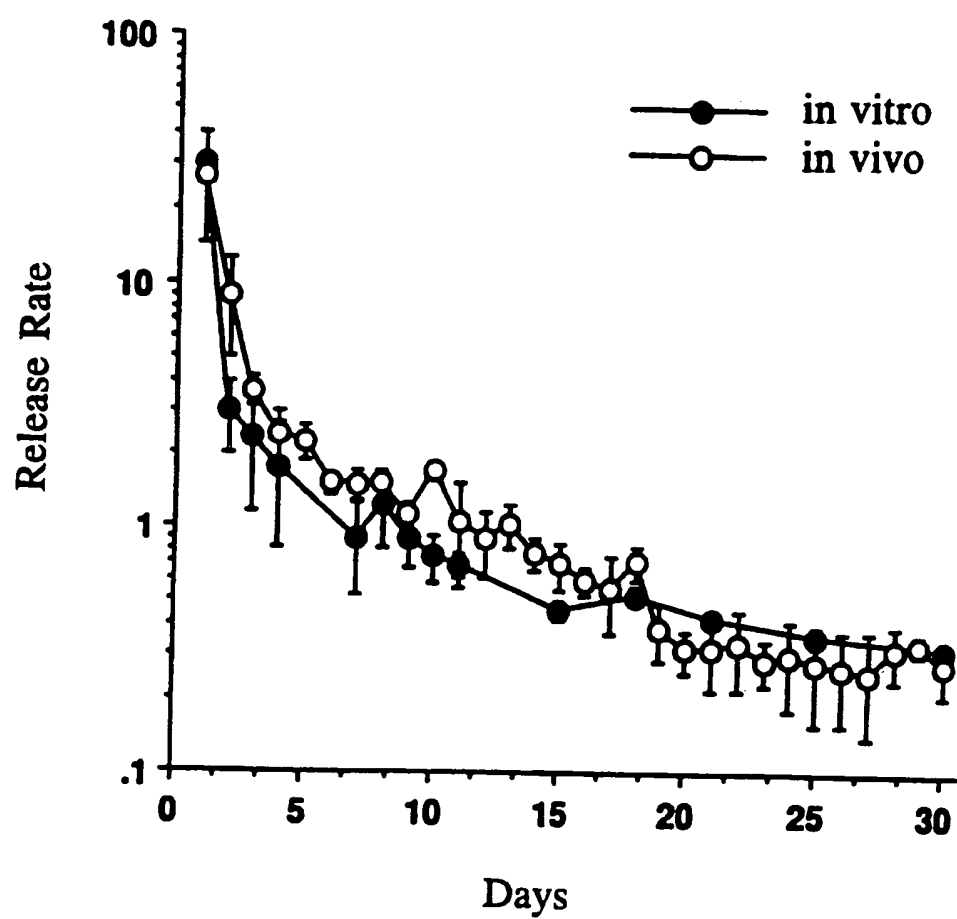


Figure 4

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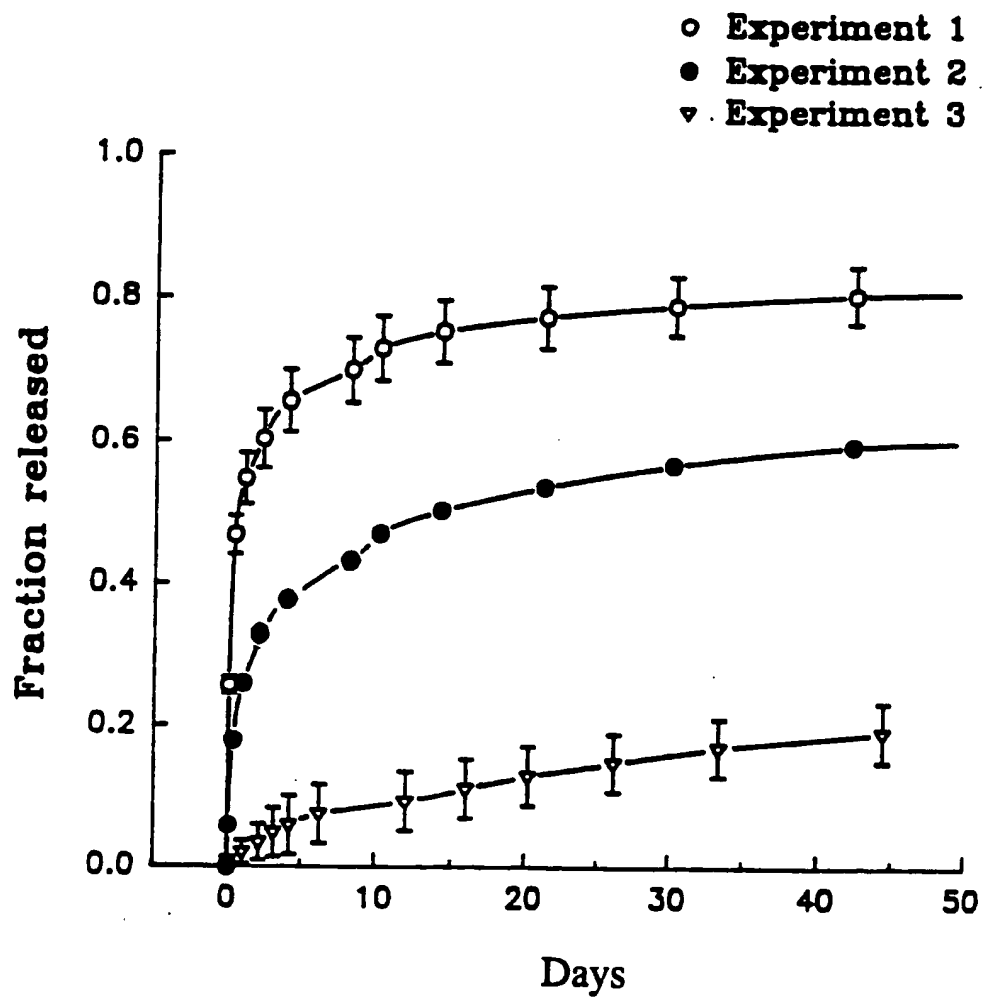


Figure 5

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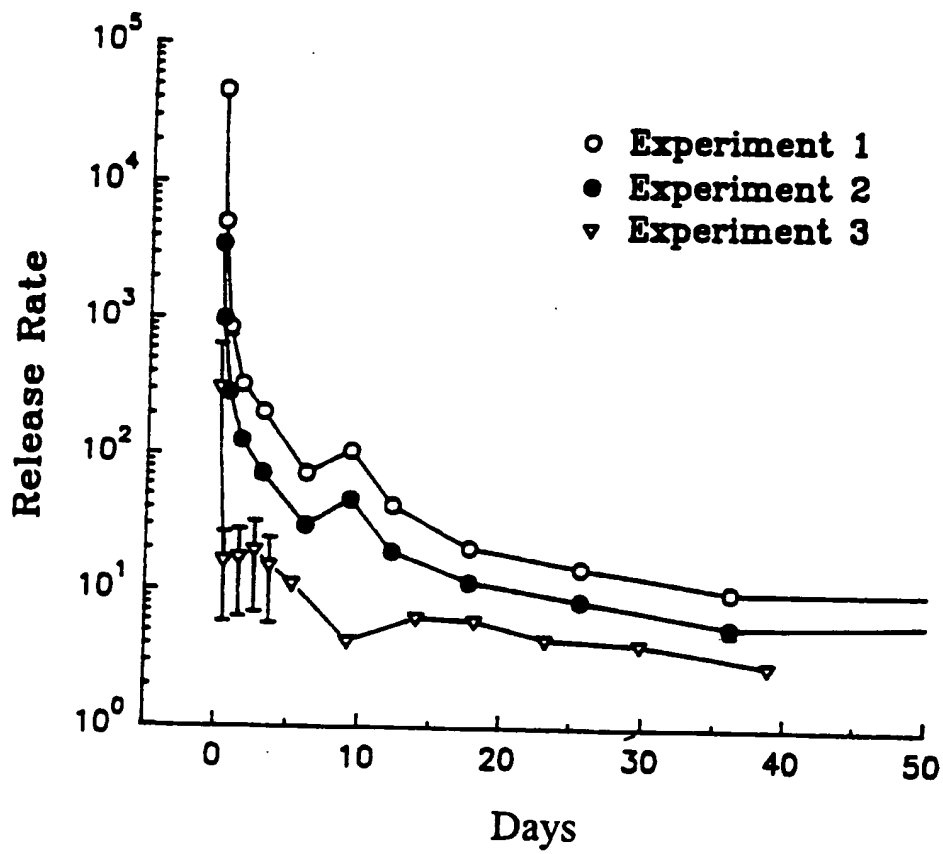


Figure 6

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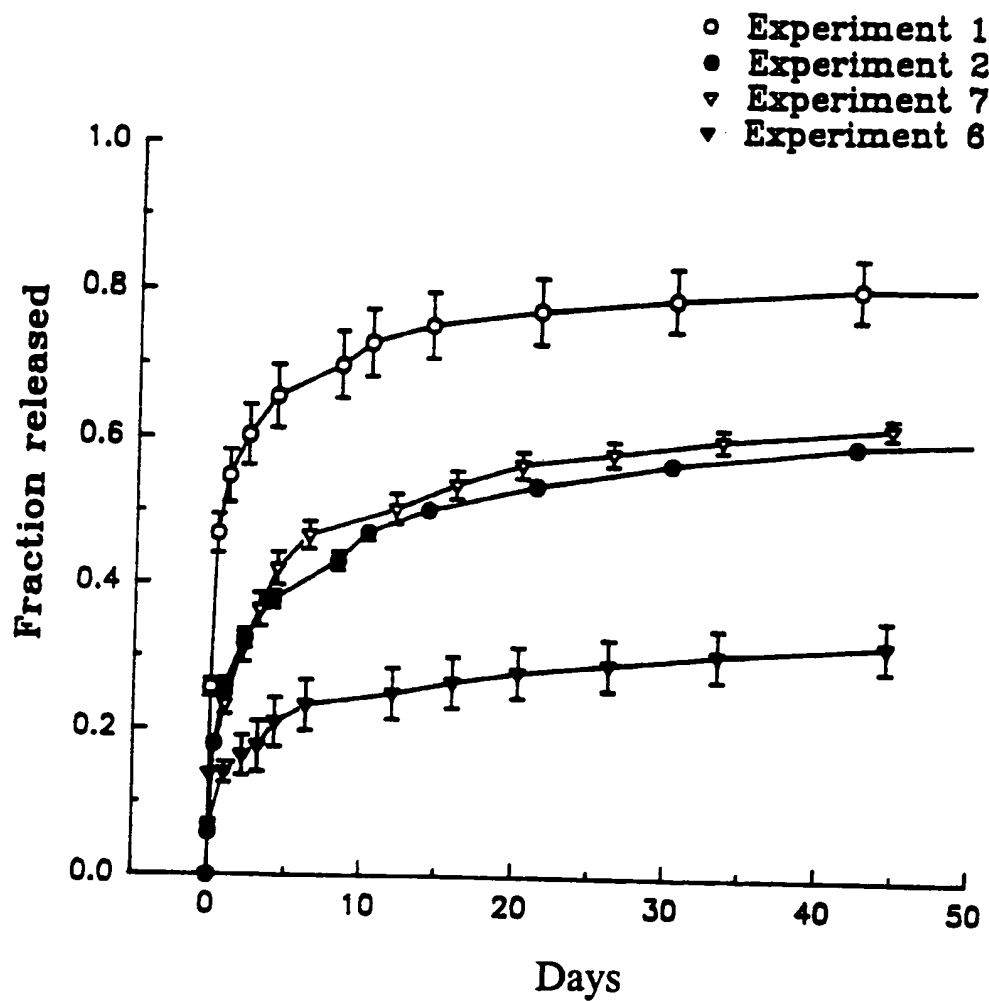


Figure 7

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/00829

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 A61K9/16		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.Cl. 5	A61K	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
Y	EP,A,0 171 603 (AKZO GMBH) 19 February 1986 & US,A,4 645 664 cited in the application ---	1-23
Y	JOURNAL OF PHARMACEUTICAL SCIENCES vol. 73, no. 9, September 1984, WASHINGTON (US) pages 1294 - 121297 L.M. SANDERS ET AL. 'controlled release of a luteinizing hormone-releasing hormone analogue from poly(d,l-lactide-co-glycolide) microspheres' see the whole document --- -/--	1-23
<sup>10</sup> Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
14 MAY 1993	02.06.93	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	BENZ K.F.	



III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	EP,A,0 133 988 (HOECHST AKTIENGESELLSCHAFT) 13 March 1985 see the whole document see page 8; examples 1,3 ---	1-3, 5-11, 16-23
Y	DE,A,4 023 134 (DEBIOPHARM S.A.) 31 January 1991 see the whole document ---	1-20
A	EP,A,0 251 476 (SYNTEX (U.S.A.) INC.) 7 January 1988 see page 16; example 7 ---	10
A	EP,A,0 245 535 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 19 November 1987 see the whole document ---	20
A	EP,A,0 388 718 (ADVANCED POLYMER SYSTEMS, INC.) 26 September 1990 see page 3, line 14 - line 33 see page 6, line 31 - line 32 see page 6 - page 7; example 1 see claim 8 -----	1-23